

WHAT IS CLAIMED IS:

1. A cDNA polynucleotide comprising a nucleotide sequence for encoding thermal hysteresis proteins derived from the *Tenebrionoidea* Superfamily.

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2. The cDNA according to claim 1, wherein said thermal hysteresis proteins are Type III anti-freeze proteins.

10 3. The cDNA according to claim 1, wherein said thermal hysteresis proteins are from the group consisting of Tm 12.86, Tm 2.2, Tm 3.4, Tm 3.9, Tm 7.5, Tm 2.3, Tm 13.17, Tm 12.84 and isoforms thereof.

15 4. The cDNA according to claim 1, wherein said nucleotide sequences are from the group consisting of SEQ ID NO.'s 2, 5, 6, 9, 12, 15, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 44-47 and their respective complements.

20 5. The cDNA according to claim 1, wherein said nucleotide sequence further includes a 5' end selected from the group consisting of non-his/signal plus, non-his/signal minus, his/signal plus and his/signal minus.

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6. A m-RNA polynucleotide comprising a nucleotide sequence for encoding thermal hysteresis proteins derived from the *Tenebrionoidea* Superfamily transcribed from said cDNA in claim 1.

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7. The mRNA according to claim 6, wherein said thermal hysteresis proteins are Type III anti-freeze proteins.

35 8. The mRNA according to claim 6, wherein said thermal hysteresis proteins are from the group consisting of Tm 12.86, Tm 2.2, Tm 3.4, Tm 3.9, Tm 7.5, Tm 2.3, Tm 13.17, Tm 12.84 and isoforms thereof.

9. The mRNA according to claim 6, wherein said nucleotide sequences comprise the fully complementary sequences from the group consisting of SEQ ID NO.'s 2, 5, 6, 9, 12, 15, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 44-47.

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10. The mRNA according to claim 6, wherein said nucleotide sequence further includes a 5' end selected from the group consisting of non-his/signal plus, non-his/signal minus, his/signal plus and his/signal minus.

11. A DNA probe having a sequence complementary or identical to a sequence of contiguous nucleotides for at least a portion of said cDNA sequences of claim 1.

5 12. A RNA probe having a sequence complementary or identical to a sequence of said nucleotides of said nucleotide sequences of claim 6.

13. A recombinant vector containing the cDNA of claim 1.

10 14. A thermal hysteresis protein derived from the *Tenebrionoidea* Superfamily which lowers the freezing point of a solution without effecting the melting point of the solution.

15 15. The thermal hysteresis protein according to claim 14, wherein said protein has an amino acid sequence from the group consisting of SEQ ID NO.'s 1 to 39 and 44 to 48.

16. A consensus sequence with a nucleotide sequence from the group consisting of SEQ ID NO.'s 44 to 47.

20 17. A consensus sequence with an amino acid sequence from the group consisting of SEQ ID NO.'s 44 to 47.

18. A consensus sequence with amino acid sequences as in SEQ ID NO. 48.

25 19. A primer having a nucleotide sequence selected from the group consisting of SEQ ID NO.'s 40 to 43.

20. A method for producing a polypeptide having antifreeze properties comprising:
forming a cloning vector with a Tm 12.86 family member gene encoding an antifreeze polypeptide;

30 transferring genes of said cloning vector into DNA of host cell to create a transformed cell;
expressing a mRNA sequence and a translated amino acid sequence from said recombinant expression vector, said sequence being isoforms of said Tm 12.86 *T.molitor* antifreeze polypeptide.

35 21. The method according to claim 20, further comprising isolating said amino acid sequence and establishing antifreeze protein activity for said amino acid sequence.

22. The method according to claim 20, wherein said amino acid sequences are from set forth SEQ ID NO.'s 2-39 and 44-48.

23. The method according to claim 20, wherein said polypeptide has an apparent molecular weight from about 11,000 to 25,000 Daltons.

5 24. The method according to claim 20, wherein said isolating said amino acid sequence comprises extraction from inclusion bodies within said transformed host bacterial cell.

10 25. The method according to claim 20, wherein establishing activity further comprises denaturing and extracting proteins from said transformed cells followed by renaturizing and purifying said polypeptide, followed by further denaturing and refolding.

15 26. The method according to claim 25, wherein said activity step provides antifreeze polypeptide activity as measured by thermal hysteresis or antifreeze specific recrystallization inhibition.

20 27. A method for providing antifreeze or recrystallization inhibition properties to a subject formulation comprising incorporating at least 0.1 micro gm to about 1 mg of an activated polypeptide into 1 mL of a subject formulation to obtain recrystallization inhibition or about 1 mg to about 25 mg of said activated polypeptide into about 1 mL of a subject formulation to thermal hysteresis.

25 28. The method according to claim 27, wherein said activated polypeptide provides a non-colligative freezing point depression and an antifreeze specific inhibition of recrystallization.

30 29. The method according to claim 27, further comprising an enhancing activator species.

35 30. The method according to claim 29, wherein said activator is an endogenous activator from *T. molitor* or Tm 12.86 antisera.

30 31. The method according to claim 27, wherein said activated protein is incorporated into plant, produce or fish in an amount sufficient to provide antifreeze protection.

35 32. The method according to claim 27, wherein said activated protein is incorporated into a region of a target tissue in an amount sufficient to provide antifreeze protein controlled limited tumor cell or target tissue cryoinjury during cryosurgery.

35 33. The method according to claim 27, wherein said activated protein is incorporated into hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic

or hypothermic preservation of cells and tissues by incorporating said protein into said cells, tissue, or cell membranes in a controlled amount sufficient to provide antifreeze protection.

34. The method according to claim 27, wherein said activated protein is incorporated into
5 de-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces.

35. The method according to claim 34, wherein said surfaces comprise road, aircraft, household products, cosmetic products, machinery and plant surfaces.

10 36. The method according to claim 27, wherein said activated protein is incorporated into a food product in an amount sufficient to provide antifreeze protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage .

15 37. The method according to claim 27, wherein the polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization.

20 38. Native endogenous Type III anti-freeze proteins from the *Tenebrionoidea* Superfamily .

39. A Tm 12.86 antibody/antiserum which is used as a screening device to identify positive recombinant plaques containing cloned inserts capable in an expression vector system to produce recombinant products recognized by the antibody/antiserum.

25 40. A Tm 12.86 antibody/antiserum which is used as a screening device to screen cDNA libraries in an expression system, including cross-species cDNA libraries to identify homologous sequences in other species.